Structure
Previews

Cutting-Hedge Research into Bacterial Invasion

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Glycoside hydrolases are the tools that pathogenic bacteria use to cut through the defensive glycan structure on host cell surfaces. In this issue of *Structure*, Pluvinage et al. (2011) report how a bacterial polypeptide with more than one hydrolase module broadens the effective substrate specificity.

It's a jungle out there. So the elite live in gated communities protected by high thorny privacy hedges, burglar alarms, and surveillance and security patrols. An invading burglar needs to have the right tools to cut a stealthy path through the hedge, being careful to not set off any alarms, or, if necessary, to inactivate a tripped alarm before "the authorities have been dispatched."

Pathogenic bacteria, like burglars, have a hard life. In order to infect their targets, they have to overcome a seemingly impenetrable "security hedge" of cell surface carbohydrates (glycans). Not only are the bushes of this hedge thickly distributed, but their branches are heterogeneous in makeup and structure. Some branches are long and extended, while others are shorter but contain multiple sub-branches with various terminal structures.

The basic tools that bacteria use to cut through the hedge are the glycoside hydrolases (GHs), enzymes that cleave the chemical linkage between the sugar building blocks that make up the glycan structures. The problem with GHs, though, is that they are specific for not just the chemical linkage itself and the substituents on either side of the linkage, but also to the stereochemistry: the relative positions of one sugar ring to the next, and sometimes to the context of the linkage within the glycan. Therefore, such bacteria have amassed an arsenal of GHs with different specificities, reflecting the variety of glycan structures that commonly occur on cell surfaces, those generated by the mammalian N-glycosylation pathway.

The paper by Pluvinage et al., (2011) in this issue of *Structure* describes structural and functional studies on one of the enzymes, StrH, that have been associated with infection by *Streptococcus pneumonia*, an important human pathogen. StrH has specificity for a linkage between N-acetylglucosamine (GlcNAc) and mannose (Man) sugar residues connected in a β-conformation between positions 1 and 2, respectively. However, such linkages can occur in different contexts within the usual core N-glycan:, they can be on an α(1-3) branch of the glycan tree (referring to the way the mannose is connected to the main trunk of the glycan), or an α(1-6) branch, with or without a further GlcNAc bisecting the two branches. Any self-respecting GH would be able to distinguish these different contexts. But it seemed initially that StrH could handle them all. However, on further inspection, the authors realized that StrH was not one, but two different yet related enzyme modules within the same polypeptide, each a member of the GH20 family. Using crystallographic structural analysis of the two GH20 catalytic modules and inactivation by site-directed mutagenesis followed by screening on glycan arrays, the authors have identified the specificities of the GH20 components.

The study of the substrate specificities of the GH20 modules was facilitated by the generation of mutant versions of each in which residues critical for enzymatic activity, but not substrate binding, were altered. These mutants were subjected to binding studies with an array of typical N-glycan structures and revealed that the first (N-terminal) GH20 unit (GH20A) is able to associate with the GlcNAc from either the α(1-3) or α(1-6) arm, whereas the second unit, GH20B, only recognizes the GlcNAc on the α(1-3) branch. However, GH20B was able to act on bisected glycans with the intervening GlcNAc, whereas GH20A was not. That two separate enzyme modules have evolved to recognize the structural glycan diversity, as opposed to a more promiscuous single domain, speaks to the nature of carbohydrate variability.

Crystallographic analyses of the inactivated mutants in complex with various substrate structures have shed light on the basis for recognition. Unlike the Golgi enzyme α-mannosidase II, which recognizes both α(1-3) and α(1-6) linkages in roughly the same orientation (Shah et al., 2008), GH20A accommodates both linkages by differentially positioning the +1 mannose (preceding the scissile bond) depending on the linkage. This positioning is mediated by a Trp residue, not present in GH20B, which forms an "aromatic clamp" in the substrate-binding cleft. Unlike mannosidase II, there is no evidence for a sequential cleavage of one linkage before the other. GH20B, in contrast, can only accommodate the α(1-3) arm. The absence of the "aromatic clamp" in GH20B, however, allows this module to interact with substrates having a bisecting GlcNAc, thus providing an elegant explanation for the requirement of both enzyme modules for full processing of the mature glycan.

While characterized in the most detail, StrH is far from unique in having evolved a multimodular arrangement to handle complicated glycan structures. The mammalian intestinal glucosidases, maltase-glucoamylase and sucrose-isomaltase have taken such an approach to the next level. As each of these gene products consists of two GH31 family modules, our digestive system has evolved four related enzymes to degrade the complex starch structures in our diet in order to derive all the nutritional glucose possible (Jones et al., 2011).

In Pluvinage et al., (2011), the authors were able to inhibit both the GH20

modules with known inhibitors of this class of enzymes, showing that their inactivation recapitulates the StrH null phenotype. An interesting question remaining is the mutual contribution of each of the GH20 domains to this function; the data presented suggest a more dominant role for GH20A. The authors also investigate a proposed additional role of StrH in infection: evasion of the immune system (inactivating the security alarm) by blocking binding of complement factor C3 to its activating protease, convertase. Even though the StrH structure was found to have a three-helix bundle domain with similarity to a complement inhibitory protein SCIN, the results reported here indicate that the inhibitors of GH activity reduce the immune protection of bacteria to a similar extent as the StrH null mutants. Thus, it is the activity of the enzyme, rather than its structural features, that is responsible for immune evasion. Perhaps the bacteria are using the cleaved branches as camouflage. A fuller understanding of this observation will have to await further study. What is evident from these results is that being able to clear a path through the security hedge of N-glycans on the host cell surface has been a significant selection force in the evolution of pathogenic bacteria.

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Fibrinogen Unfolding Mechanisms Are Not Too Much of a Stretch

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Molecular explanations for the extraordinary elasticity and extensibility of fibrin fibers are still lacking. Now, Zhmurov et al. (2011) use force spectroscopy experiments, and innovative simulations that match the time and force scales of these experiments, to study fibrinogen behavior under an applied force providing deeper insights into this process.

Fibrinogen is a hexameric plasma protein composed of a pair of three peptide chains designated Aα, Bβ, and γ. At each end of the protein are the globular D regions comprised of the β and γ nodules. Triple α-helical coiled coils connect the D regions to the central, globular E region, which contains the two pairs of fibrinopeptides A and B. Not visible in the crystal structure are the 389 residues long, flexible α-C regions (Figure 1A) (Kollman et al., 2009).

In hemostasis, activated thrombin removes fibrinopeptides A and B, thereby exposing the A and B knobs in the central E region and converting fibrinogen to fibrin. Fibrin then assembles spontaneously into two-stranded, half-staggered protofibrils (Figure 1B). The key interaction that directs this assembly is the A:a interaction between the A knob and the a pocket in the γ nodule. The protofibrils then assemble radially into about 100 nm wide fibrin fibers that comprise the major structural component of a blood clot. The key interactions of protofibril assembly are thought to be the B:b interaction between the B knob and b pocket in the β nodule. Additionally, there is increasing evidence that the α-C region plays a critical role in protofibril assembly (Ping et al., 2011).

Fibrin fibers are among the most elastic and extensible protein fibers (Liu et al., 2006, 2010). They can be stretched elastically to nearly twice their length and to 2.5 times their lengths before rupturing. This large, elastic extensibility was unexpected because fibrin fibers assemble in a regular, near-crystalline fashion, and most protein fibers that have this regular structure, such as collagen fibrils, actin filaments, and microtubules, are far less extensible. The apparent contradiction between large, elastic extensibility and regular, crystalline structure could be resolved by identifying flexible linkers between fibrin molecules and protofibrils, or regions on the fibrinogen molecule that could stretch or unfold.

The origin of this elastic extensibility has been a topic of debate over the past few years, and experimental and computational studies implicate the following possible sources: (1) unfolding of the α-helical coiled coils into β strands (Brown et al., 2007; Lim et al., 2008); (2) unfolding of the γ nodules (Averett et al., 2008); and (3) unfolding of the α-C region (Houser et al., 2010) (Figure 2). A complete understanding of the mechanical